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PERIODICITY IN THE PRODUCTION OF MALES IN *HYDATINA SENTA*.¹

A. FRANKLIN SHULL.

INTRODUCTION.

There is often a well-marked rhythm in the production of males in the rotifer *Hydatina senta*. Generation after generation may pass with few or no male-producing females; while later, in a few successive generations, male-producers may be abundant, only to be succeeded by a period in which male-producers are uncommon or wanting. Although this rhythm has not been mentioned by all students of the life cycle of *Hydatina*, and has been emphasized by few of them, it can hardly have escaped notice by any one who has bred this species for several months. In another genus, *Asplanchna*, Mitchell (1913) has laid stress upon this rhythmical appearance of males, as a basis for certain theoretical conclusions, and has called attention, by way of generalization, to the same rhythm in *Hydatina*.

Regarding the cause of this periodicity, there is not general agreement. Mitchell, writing of *Asplanchna* but extending his conclusions to rotifers in general, appears at times to regard the rhythm as the effect of an internal factor, and again as due to environmental conditions. He says "this rhythm is not the result of external conditions" but "is not absolutely independent of them."² Later he adds that "male production . . . is a matter of physiological potential and under the more or less direct control of nutrition" (p. 229). At other places he states that "male production . . . is a phenomenon all but wholly under nutritive control . . ." (p. 246); "these male and non-male producing strains . . . exist . . . and . . . these strains are also produced by nutritive changes" (p. 247); and "qualitative and quantitative changes in nutrition will be found the universal sex-controlling factors in this group" (the rotifers)

¹ Contribution from the Zoölogical Laboratory of the University of Michigan.

² *Op. cit.*, p. 228.

(p. 253). Whitney (1914), on the other hand, has no hesitancy in ascribing the periodicity of male production to external factors; thus, in mentioning earlier work of his own, "he was of the opinion that whatever the potent factor was that sometimes caused only females to be produced, and at other times caused nearly all males to be produced, it must be an external factor." On the basis of his recent work he attributes this periodicity to alternation of the active and quiescent states of the protozoan food of the rotifers.

In my own work on *Hydatina* during several years, certain lines have been bred so long, and so many families completely reared, that further light may be thrown upon the rhythm of male production. In the following pages evidence is first presented, bearing on the regularity of the periodicity of male production, and the probable independence of this periodicity of the environment. Later the supposed evidence that male production is correlated with nutrition is discussed.

REGULARITY OF THE PERIODICITY OF MALE PRODUCTION.

Conditions Necessary to the Demonstration of Periodicity.

In parthenogenetic lines that produce many males, rhythm is not as easily demonstrated as in lines producing few males. If the proportion of males in a line producing many males be represented by a curve, there are so many irregularities in it, so many minor humps even in the periods of few males, that the larger humps are less striking. If the lines produce few males, on the other hand, the whole curve may be so lowered that the periods of depression are below the base line; that is, there are no males at all in these periods. In such cases the rhythm may be quite striking.

Further difficulty in detecting rhythm is introduced by rearing only one family in each generation. There are great individual differences between families taken at the same period, so that the family chosen may or may not be an average of all families that might be reared at the same time. Furthermore, the method of publishing the results, merely giving the total for the one family in each generation, often makes the rhythm appear less definite; because it often happens that in three or four successive

generations, in a period of many males, the males are produced mostly by the daughters at the end of the family in the first of these generations, by the daughters in the middle of the family in the second generation, and by the daughters in the first part of the family in the third generation. In the manner in which these results have been published, the males of the first and third generation (appearing under date of the beginning of their respective families) seem to be four or five days apart, whereas they may be only one day apart, or even hatched on the same day.

It seemed advisable to obviate as many of these difficulties as possible, and to obtain evidence of periodicity less open to objection. The method adopted was as follows: First, a line was selected which was producing only a moderate number of males, with the expectation that the periods of male production would be completely separated by periods in which there were no males at all. Second, instead of isolating all the daughters of a single family in each generation, parts of a number of families were isolated. This second precaution was taken to smooth out irregularities and to reduce the *apparent* length (and hence the overlapping) of the periods, both of many and of few males.

Lines Exhibiting Periodicity.

The line selected to meet the above conditions was one of the F_2 lines from a cross between an English and a Nebraska line, described in an earlier paper (Shull, '15). Several females of this line, all of approximately the same age, were placed together in a dish. When they reached maturity, the daughters of the early part of their families were rejected; but the daughters produced after about 24 hours of egg laying were preserved. From these daughters the "sex ratio" (ratio of male-producing to female-producing females) was determined, and from them also the next generation was reared. The first daughters were in each case rejected because, as I have shown before (Shull, '10), the first daughters are less commonly male-producers than are daughters in the middle of the family.

The daughters selected for rearing the next generation were in like manner kept together in one dish until about 24 hours after their first daughters appeared. At this time a second lot, all of

about the same age, was isolated to determine the sex ratio of the second generation, and so on.

From 30 to 100 daughters, all of nearly the same age, and from the same parts of their respective families (end of first day of egg-laying) were recorded for each generation. A new generation was, by this method, secured every three days, as a rule, instead of every two days, as is possible when the first daughters of a family are used as parents of the next generation.

The number of male-producing and female-producing females in each generation thus recorded is given in Table I. Male pro-

TABLE I.

SHOWING THE NUMBER OF MALE-PRODUCING ($\sigma^7 \varphi$) AND FEMALE-PRODUCING ($\varphi \varphi$) FEMALES IN A LINE OF *Hydatina senta*.

The male-producers occur periodically.

Date.	Number of $\sigma^7 \varphi$.	Number of $\varphi \varphi$.	Date.	Number of $\sigma^7 \varphi$.	Number of $\varphi \varphi$.
Jan 16	2	24	Mar. 14	0	44
19	0	24	17	0	38
22	1	35	20	0	42
25	3	33	23	0	23
28	19	46	26	8	40
30	14	46	29	36	48
Feb. 2	0	56	Apr. 1	7	71
5	0	40	4	1	51
9	2	38	7	0	37
12	0	43	10	0	40
15	0	47	13	1	36
18	0	48	16	0	35
21	0	13	19	0	43
24	0	35	22	3	85
27	17	21	26	68	51
Mar. 2	13	27	29	32	23
5	6	29	May 2	18	34
8	2	36	5	3	50
11	0	42	8	0	44

duction in this line, when bred as described above, showed a well-marked periodicity, the interval being about one month. Conditions were purposely kept fairly uniform; but even if it were not possible to prevent changes of the medium, it is scarcely probable that any external condition favoring male production should have recurred with such regularity. Moreover, were this periodicity due to external factors, the intervals between periods of male production should be the same in all lines bred at the same time and subjected to the same conditions. That this was not the case will now be shown.

In another line, bred for a much longer time than was the line described above, there was equally clear evidence of regular periodicity, though the extent of the waves of male production was not determined. A line of rotifers obtained from England in the fall of 1912, described in another paper (Shull, '15), has been reared up to the present time. No considerable numbers of individuals of this line have as a rule been *isolated*, hence the sex ratio can not be stated; but during the time when the periodicity of other lines was being examined, several dozen

TABLE II.

SHOWING DATES BETWEEN WHICH MALES WERE PRODUCED IN AN ENGLISH LINE OF *Hydatina senta*.

No males appeared between one period of male production and the next.

Number of Period of Male Production.	Dates Between Which Males Were Found.
First.....	February 13 to 25, 1913
Second.....	April 17 to 26, 1913
Third.....	June 19 to 24, 1913
Fourth.....	August 24 to 30, 1913
Fifth.....	October 31 to November 15, 1913

individuals of most generations were reared in two or three dishes. As the number of males was always small in this line, males were found in these "mass" cultures only occasionally. They nearly always appeared in the dishes containing several successive generations, and then were wanting for a considerably longer period. There was thus a rhythm of male production, which, as shown in Table II., proved to be fairly regular and definite, though the number of male-producers in each period was not known.

The interval between periods of male production was, in this line, a trifle over two months. Inasmuch as this line was bred in part simultaneously with the one recorded in Table I., the difference in the interval of male production in the two lines (one month in the first, over two months in the second) effectually disposes of any suspicion that this rhythm was induced by external conditions.

A third line which showed evidence of periodicity in the production of males was obtained from Nebraska in 1912, and has been reared ever since. This line is also described in a recent

paper (Shull, '15). Complete families were isolated from early in November to December 15, 1912, but no males appeared. During the time when periodicity of male production was being studied, representative mass cultures were reared. These cultures showed males in small numbers only, and at times separated by wide intervals. The times of male production, as far as known, are shown in Table III., though the proportion of males was not recorded.

The periods of male production in the Nebraska line are separated by intervals of three to five months, the interval

TABLE III.

SHOWING TIMES AT WHICH MALES WERE PRODUCED IN A NEBRASKA LINE OF
Hydatina senta.

Number of Period of Male Production.	Dates Between Which Males were Found.
First.....	January 15 to February 1, 1913
Second.....	April 22, 23, 1913
Third.....	August 10 to 16, 1913
Fourth.....	December 7 to 11, 1913
Observation wanting ¹	
Sixth(?) ²	November 1, 1914

increasing with the age of the line. Though no complete families were reared, during any of these "waves" of male production, it was evident that in the later periods there were fewer males than in the earlier ones.³

In each of the three lines described, there was a well-marked rhythm in the production of males. The great regularity of this periodicity, especially in the first two of these lines, and the fact that the lines differed considerably from one another in regard to the interval between periods of male production, forbid the assumption that the waves of male production were brought on by specific external conditions.

¹ Observations were wanting from March 8 to June 10, 1914. Probably not more than one period of male production fell between these dates, hence the next males recorded are to be regarded as belonging to the sixth period.

² A single male-producing female appeared in this "wave" of male production.

³ In the English line described in Table II., while the intervals between periods of male production did not increase, with the age of the line, the number of males in successive periods plainly decreased. This is a confirmation of a conclusion which I formerly drew from lines bred through shorter periods, namely, that the proportion of male-producing females gradually decreases with the age of the line. Whether, as Mitchell suspects, this decrease is due to uniformity of conditions, is a question not answered by the evidence.

No statement here made is to be construed, however, as a contradiction of my former claim that external conditions may alter the *extent* of male production.¹ Few biological facts are more firmly established than that external factors modify the life cycle of *Hydatina*. The results described above merely show that, under fairly uniform conditions, there is nevertheless a periodicity in the production of males which must be due to internal factors.

PERIODICITY AND NUTRITION.

Mitchell ('13) has pointed out that in *Asplanchna* periods of male production are also often, perhaps usually, periods of vigorous growth and rapid reproduction; and he concludes therefrom that male production is a result of high nutrition. This conclusion may be correct, but it is scarcely logical, since coincident events are not always related to one another as cause and effect. But assuming as Mitchell does that size of family is a guide to nutrition, let us examine all the sources of information that are extensive enough to be of value, to determine, if possible in this way, the relation of nutrition to male production. In my own work in the past few years, there have been two lines in which hundreds of families have been reared. By collecting all of the families of the same size in a single line, and recording the proportion of male-producers, it should be possible to discover to what extent size of family and male production are correlated. Obviously one must not collect in the same group families belonging to two or more unrelated lines, for one of these lines may have larger families, and at the same time (but from other causes) either many or few male-producers, so that the groups of families of large size would have on the average a correspondingly high or low proportion of male-producers. Such an apparent correlation would have no significance. Within a single line, however, no such error could affect the results. The two tables herewith presented (IV. and V.) are each compiled from families belonging to a single line.

¹ My discovery several years ago of internal differences between parthenogenetic lines of *Hydatina senta*, the result of which is a different proportion of male producers in each line, is characterized by Mitchell as a "return to the position of Punnett." Since Punnett never found an effect of external conditions, and since I never repudiated my experiments proving the effect of external conditions, there can have been no "return."

high nutrition (coupled with irregularities of nutrition). How far his statement that the male-producing females are in the larger families is correct, may be seen from Table VI., in which

TABLE V.

SHOWING SIZE OF FAMILY AND PROPORTION OF MALE-PRODUCING ($\sigma^7 \varphi$) AND FEMALE-PRODUCING ($\varphi \varphi$) FEMALES IN A SINGLE LINE OF *Hydatina senta*, DISTINCT FROM THAT IN TABLE IV.

Size of Family.	Number of Families.	Number of $\sigma^7 \varphi$.	Number of $\varphi \varphi$.	Percentage of $\sigma^7 \varphi$.
1 to 5	12	2	38	5.0
6 to 10	17	13	121	9.7
11 to 15	19	67	183	26.8
16 to 20	29	105	415	20.1
21 to 25	32	152	587	20.5
26 to 30	17	48	418	10.3
31 to 35	22	149	577	20.5
36 to 40	15	113	463	19.6
41 to 45	18	76	704	9.7
46 to 50	19	192	716	21.1
51 to 55	8	106	314	25.2

the families of the starved line in the experiment referred to (Shull, 1910, Table III.) are tabulated. The group of families containing 1 to 5 daughters, and that of families numbering 41 to 45, are omitted because there is but one family in each group.

TABLE VI.

SHOWING SIZE OF FAMILY AND PROPORTION OF MALE-PRODUCING ($\sigma^7 \varphi$) AND FEMALE-PRODUCING ($\varphi \varphi$) FEMALES IN A STARVED LINE OF *Hydatina senta* DESCRIBED IN A FORMER PAPER.

The greatest proportion of male-producers is in families of medium size.

Size of Family.	Number of Families.	Number of $\sigma^7 \varphi$.	Number of $\varphi \varphi$.	Percentage of $\sigma^7 \varphi$.
6 to 10	7	7	49	12.5
11 to 15	3	6	34	15.0
16 to 20	6	39	66	37.1
21 to 25	8	88	101	46.5
26 to 30	16	172	273	38.6
31 to 35	8	96	169	36.2
36 to 40	2	14	64	17.9

In this table it appears that the greatest number of male-producing females is found, not in the largest families, but in those of medium size. It may also be recalled that the distribution of the male producers with regard to size of family, in these starved families where nutrition was *known* to have been variable,

is not the same as the distribution in the well-fed families of Tables IV. and V., about whose nutritive conditions we know only that which size of family tells us. The argument that the numerous male-producing females of the starved line were produced as a result of high nutrition of their parents, loses much of its weight when it is shown that these male-producers were not chiefly in the largest families.

It is not to be asserted that nutrition has no effect upon male production. Indeed, Whitney ('14) has presented new evidence that *qualitative* differences of nutrition do affect male production. It is not clear what relation Whitney's results have to the question of *periodicity* of male production, whether changes of nutrition can be made to destroy the rhythm, or wholly to alter the interval, or merely to modify the extent of male production. My own starvation experiments, referred to above, left the intervals between the periods of male production unaltered, but the waves of male production and the intervening periods of female production were rendered less striking. I attributed the effects shown in these experiments to the chemical nature of the medium, and not to nutrition. Until experimental evidence indicates the contrary to be true, it is safest to assume that nutrition also, when it affects male production at all, does not alter the interval between periods of male production, but merely the extent of male production.

To summarize: Three lines of *Hydatina*, bred through many months, showed fairly regular periodicity in the production of males. One line exhibited relatively abundant male production every month; another every two months; while in the third the interval varied from three to five months during the period of observation. The fact that the interval between the periods of many males is quite regular in some lines, and is not the same in all lines reared simultaneously, indicates that this periodicity is due to an internal factor. Hundreds of families were examined to determine whether the largest families, which were presumably offspring of the best nourished parents, contained the greater number of male producing females, as Mitchell assumes they do. In well nourished lines there is some doubt whether there was any correlation between size of family and number of male

producers; in starved families, on the other hand, the greatest numbers of male producers were not in the largest families, but in those of medium size. On the statistical evidence as a whole, the influence of quantity of nutrition upon male production is held to be "not proven." When qualitative differences in nutrition affect male production, the interval between periods of many males probably remains unchanged.

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